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BETA ADRENERGIC AND ANTIARRHYTHMIC EFFECT OF APAMIN, A COMPONENT OF BEE VENOM

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DEPARTMENT OF THE ARMY
EDGEWOOD ARSENAL
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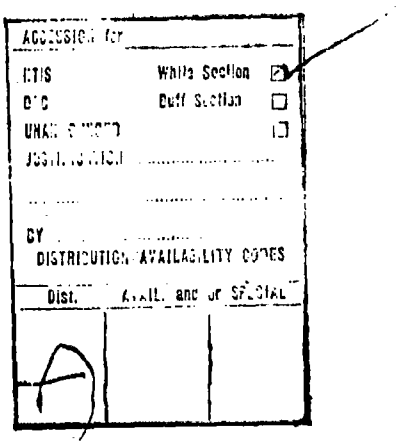

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13. ABSTRACT This study was prompted by the observation that whole bee venom produces pathophysiological changes much like those caused by certain anticholinesterase agents. During an earlier study definite cardiovascular alterations were observed which did not appear consistent with pure neuromuscular blockade. On closer analysis it was determined that both whole bee venom and one of its major components (apamin) have definite cardiostimulating properties that are described in this report. Apamin, a polypeptide, is one of three active components of bee venom having a molecular weight of 3,700. Its effects were studied in a series of 30 isolated perfused Langendorff heart preparations, and in 12 intact animals. When injected into the coronary circulation of the perfused heart, apamin produces a 50 ± 10% increase in rate, a 150 ± 50% increase in force of contraction and no change in coronary vascular resistance. These effects lasted for the duration of the experiment. In those hearts in which intrinsic arrhythmias are present a dose of 0.2 mg of apamin produced an immediate restoration of normal cardiac rhythm in addition to the effects on rate and force. In the intact monkey preparation this same dose of apamin produces an increase in heart rate, aortic blood flow and right ventricular force. There are no significant changes in arterial blood pressure, central venous pressure or cortical activity. In addition apamin appears to be nontoxic (LD50 = 15 mg/kg) when compared to whole bee venom (LD50 = 3.5 mg/kg). It would appear from these studies that apamin is a potent, nontoxic beta adrenergic-like stimulant not entirely blocked by 1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol and possessing definite antiarrhythmic properties.

14. KEYWORDS

Bee venom	Antiarrhythmic	Toxin
Cardiac stimulant	Cardiovascular	Beta adrenergic

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FOREWORD

The work described in this report was authorized under Task 1W662710AD2502, Medical Defense Against Chemical Agents, Prophylaxis and Therapy for Lethal Agents. This work was started in July 1971 and completed in March 1972.

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care" as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences – National Research Council.

DIGEST

This study was prompted by the observation that whole bee venom produces pathophysiological changes much like those caused by certain anticholinesterase agents. During an earlier study definite cardiovascular alterations were observed which did not appear consistent with pure neuromuscular blockade. On closer analysis it was determined that both whole bee venom and one of its major components (apamin) have definite cardiostimulating properties that are described in this report.

Apamin, a polypeptide, is one of three active components of bee venom having a molecular weight of 3,700. Its effects were studied in a series of 30 isolated perfused Langendorff heart preparations, and in 12 intact animals. When injected into the coronary circulation of the perfused heart, apamin produces a 50 ± 10% increase in rate, a 150 ± 50% increase in force of contraction and no change in coronary vascular resistance. These effects lasted for the duration of the experiment. In those hearts in which intrinsic arrhythmias are present a dose of 0.2 mg of apamin produced an immediate restoration of normal cardiac rhythm in addition to the effects on rate and force. In the intact monkey preparation this same dose of apamin produces an increase in heart rate, aortic blood flow and right ventricular force. There are no significant changes in arterial blood pressure, central venous pressure or cortical activity. In addition apamin appears to be nontoxic (LD50 = 15 mg/kg) when compared to whole bee venom (LD50 = 3.5 mg/kg). It would appear from these studies that apamin is a potent, nontoxic beta adrenergic- like stimulant not entirely blocked by propranolol* and possessing definite antiarrhythmic properties.

* 1-(Isopropylamino)-3-(1-naphthyloxy)-2-propanol

CONTENTS

BETA ADRENERGIC AND ANTIARRHYTHMIC EFFECT OF APAMIN, A COMPONENT OF BEE VENOM

I. INTRODUCTION.

The venom of the honey bee (Apis mellifera) has just recently been spearated into its chemical components.[1,2] The most toxic components of venom appear to be phospholipase A, and melittin, a polypeptide, both neuromuscular blocking agents which produce their lethal effect through respiratory paralysis,[3] not unlike those pathophysiological changes observed after certain anticholinesterase agents. Phospholipase A and melittin account for 85 percent of the total chemical constituents of the bee venom. The remaining main fraction of bee venom contains a relatively nontoxic substance called apamin, a polypeptide, which is thought to be no more than 2 percent of the total content of the venom.[4] Notwithstanding, apamin, like melittin and phospholipase A, was tested for physiological pharmacological activity and in addition to being less toxic than either of the other two, apamin exhibited some interesting cardiovascular effects which did not appear consistent with pure neuromuscular blockade.

This study deals with the identification of the cardiovascular effects of apamin in both dogs and monkeys.

II. MATERIALS AND METHODS.

The whole bee venom was dissolved in 0.1M NH_4CHO_2 at pH 4.5, filtered through a 0.45μ millipore filter and separated into its major component on a Sephadex G75-120 column, 2 X 30-cm, at a flow of 0.031 ml min^{-1} cm^{-2}. The apamin fraction was subsequently isolated, lyophilized and purified on a G25-80 Sephadex column, 2 X 300-cm, at a flow of 0.028 ml min^{-1} cm^{-2}. The identity of the apamin was established by amino acid analysis.

A. Isolated Perfused Dog and Monkey Hearts.

Twenty adult mongrel dogs and 10 adult rhesus monkeys were used in the initial portion of the study. Following anesthesia with 30 mg/kg Na pentobarbital I.V. the dogs and monkeys were heparinized with 100 units Na heparin per kg body weight. The animal was then quickly exsanguinated through a catheter placed in the femoral artery and advanced into the distal aorta. This blood was used to prime the reservoir used to perfuse the isolated heart. The chest of the

[1]Vick, J. A., and Shipman, W. H. Effects of Whole Bee Venom and Its Fractions (Apamin and Melittin) on Plasma Cortisol Levels in the Dog. Toxicon 10, 100-106 (1972).

[2]Haberman, E. Recent Advances in Pharmacology of Toxins. Proc. 2nd Inter. Pharmacol. Meetings, Pergamon Press, Oxford (1963).

[3]Slotta, K. H., and Vick, J. A. Identification of the Direct Lytic Factor from Cobra Venom as Cardiotoxin. Toxicon 6, 167-173 (1969).

[4]Shipman, W. H., and Cole, L. J. A Surfactant Bee Venom Fraction. Anal. Biochem. 29, 490-497 (1969).

animal was opened using a midline incision and all major branches of the aorta ligated. The heart was quickly removed from the chest, the brachiocephalic trunk catheterized, and the heart perfused with its own blood using a Sigma motor pump. Following a 30-min equilibration period a Walthen Brodie strain-gauge arch was sutured to the left ventricle to record force of contraction. Coronary perfusion pressure was monitored with an open-ended catheter inserted into the inflow side of the perfusion circuit. EKG and heart rate were recorded using a pair of needle-tipped electrodes placed in the myocardial tissue of the right and left ventricles. All recordings were made on a Grass Model 7 polygraph. Apamin was injected directly into the blood perfusing the heart and was allowed to recirculate for the duration of the experiment.

Two dog and two monkey hearts were pretreated with propranolol (0.5 mg/kg).

B. Intact Animal Experiments.

Six adult mongrel dogs and six adult rhesus monkeys were anesthetized with Na pentobarbital (30 mg/kg) and appropriate instruments used for recording of physiological change. Arterial and venous blood pressure, right ventricular pressure, aortic blood flow, EKG, EEG, heart rate and respiratory rate were continuously and simultaneously recorded on a Grass Model 7 polygraph in a manner previously described. Thirty minutes after instrumentation graded doses of apamin (10 μg/kg - 0.1 mg/kg) dissolved in sterile saline were injected into the animals. All injections were made into the femoral vein.

III. RESULTS.

A. Isolated Hearts.

Doses of 0.2 mg apamin injected directly into the coronary circulation of the isolated perfused dog heart produced a 50 ± 10% increase in rate, no change in coronary perfusion pressure and a 150 ± 50% increase in force (figure 1). In the monkey hearts a 65 ± 8% increase in rate and a 130 ± 20% increase in force was observed with no change in coronary perfusion pressure. These changes were observed within 3 to 5 minutes following injection and lasted for approximately 30 minutes at which time a gradual decrease in both rate and force occurred, stabilizing at levels well above control for the duration of the experiment (90 minutes).

In approximately 50% of the dog and monkey hearts intrinsic arrhythmias were noted immediately following removal of the heart from the chest of the animals, a problem which appears to be inherent to the perfusion procedure. Apamin in the dose described above produced an immediate restoration of normal cardiac rhythm. This apparent antiarrhythmic action lasted through the entire course of the experiment and was in addition to the observed effects on rate and force. Figure 2 shows the typical response of the isolated heart to 0.2 mg apamin. The striking antiarrhythmic effects as well as the increase in force and rate are apparent.

In the two dog and two monkey hearts pretreated with propranolol the increase in force and rate previously observed were attenuated but not eliminated. Figure 3 shows one experiment typical of the decreased response of the heart to apamin following propranolol.

An antihistaminic (Benadryl), an antiserotonin (LSD) and an alpha adrenergic blocking agent (phenoxybenzamine) did not alter the response of the heart to apamin.

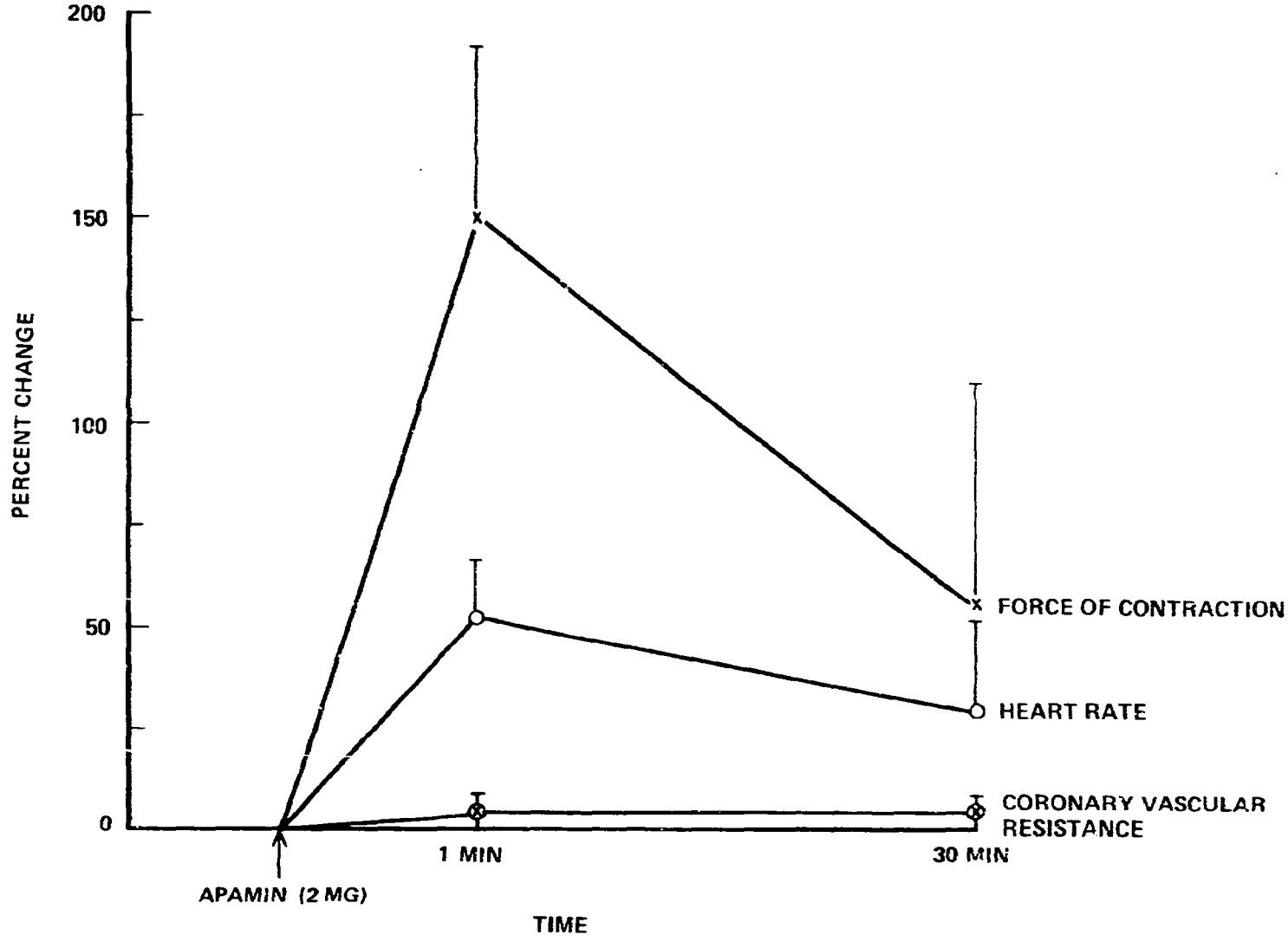

Figure 1. Effect of Apamin on Force of Contraction, Heart Rate and Coronary Vascular Resistance in the Isolated Perfused Heart Preparation.

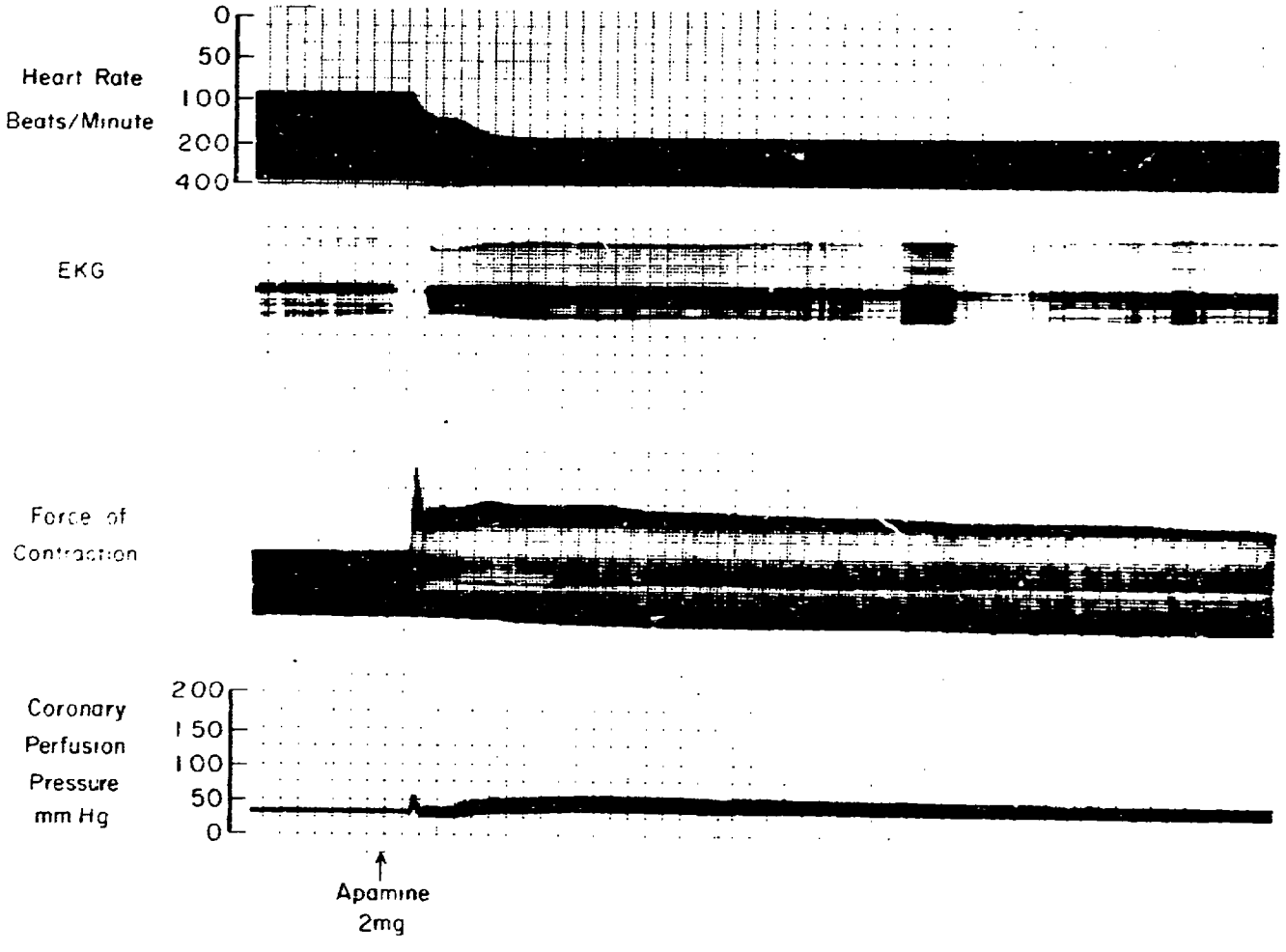

Figure 2. Effect of Apamin on Heart Rate, EKG, Force of Contraction and Coronary Perfusion Pressure in the Isolated Heart Preparation.

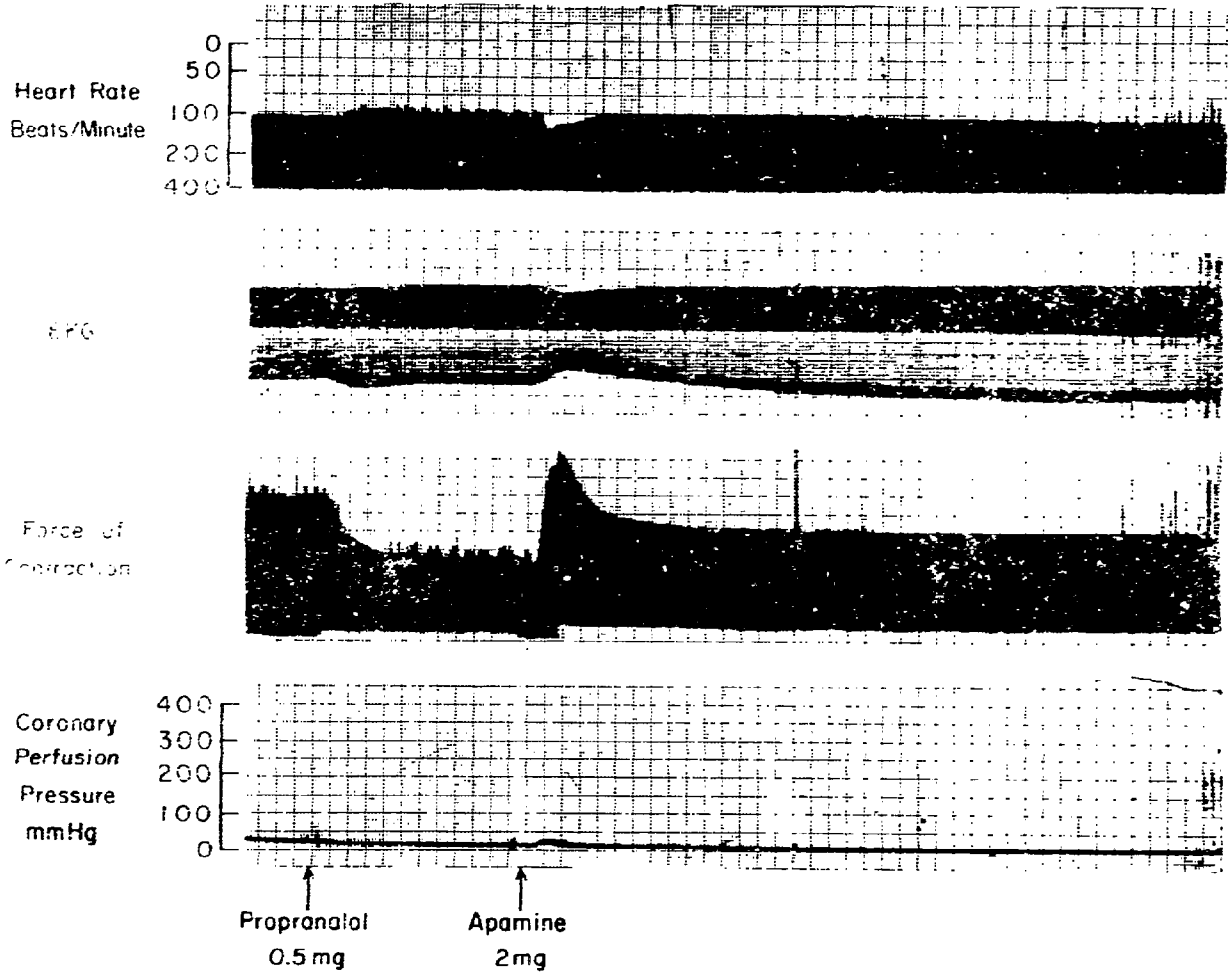

Figure 3. Effect of Apamin After Pretreatment With Propranolol in the Isolated Perfused Heart Preparation.

B. Intact Animals.

Doses of from 10 μg/kg to 0.1 mg/kg apamin produced a 35 ± 15% increase in heart rate, a 40 ± 25% increase in aortic flow and a 50 ± 40% increase in right ventricular pressure. No significant change in arterial or venous blood pressure, EKG, or cortical electrical activity (EEG) were noted in any of the animals. A slight, but not significant (10 ± 8%) decrease in arterial pressure was observed which was apparently due to reflex vasodilatation.

IV. DISCUSSION.

Apamin is a relatively nontoxic (LD50 = 15 mg/kg in mice) component of whole bee venom when compared to phospholipase A (LD50 = 1.5 mg/kg) melittin (LD50 = 2.5 mg/kg) or the whole been venom itself (LD50 = 3.5 mg/kg). In addition to being free of overall toxicity, the apamin, unlike the other components, possesses strong beta adrenergic-like activities. The well-known beta response of the heart is closely mimicked by low doses of apamin. Significant and long-lasting increases in both rate and force are observed without any observed change in coronary perfusion pressure. In that flow in the isolated heart preparation remains constant, a lack of change indicates that this drug does not affect the overall resistance in the coronary vasculature. $R = \frac{P}{F}$, meaning that when *force* is constant *resistance* is directly related to *pressure*. Thus the increase in rate and force noted with apamin is not assocuated with either a coronary vasoconstriction or vasodilatation. This observation is unique to beta adrenergic drugs in that most cause the coronary vascular resistance of the heart to increase.

The observed antiarrhythmic action of apamin was of great interest and closely mimicks the effect of isoproterenol on the heart. In this preparation, the effect of apamin is sustained for 90 minutes, however, while that of a single injection of isoproterenol is relatively short (5 - 10 min). It might very well be that apamin could be studied for long-term antiarrhythmic properties in other experimental preparations. In studies currently underway in our laboratory, the effects of apamin in the intact animal are being applied to various hemorrhagic and endotoxin shock models in an attempt to support both cardiac function and peripheral vascular flow to vital tissues. Early indications are that apamin is capable of supporting a decreased cardiac work and preventing a generalized cardiovascular collapse.

LITERATURE CITED

1. Vick, J. A., and Shipman, W. H. Effects of Whole Bee Venom and Its Fractions (Apamin and Melittin) on Plasma Cortisol Levels in the Dog. Toxicon 10, 100-106 (1972).

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